A MODIFICATION OF THE McCRADY METHOD OF THE NUMERICAL INTERPRETATION OF FERMENTATION-TUBE RESULTS*

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In the recent report of the American Public Health Association, issued in 1917, on the standard methods of bacteriologic water analysis, it is recommended that the numerical interpretation of fermentation-tube results in the determination of B. coli and allied bacteria should be based on the method originally outlined by Phelps.[1] More exact procedures of arriving at the most probable number of bacteria from qualitative results only were not favorably reviewed in the above report, since their application "to a correct mathematical solution of any considerable series of results involves mathematical calculations so complex as to be inpracticable of application in general practice."

Among the methods proposed for such numerical interpretations is the one described and completely analyzed by McCrady.[2] McCrady bases his consideration of the problem on the fact that "the frequency of the appearance of particular fermenting organisms is an exponential function of the number of such organisms in the sample tested and that every fermentation-tube result, whether simple or compound, corresponds to one most probable number of organisms." In his discussion, he makes use of a general probability formula, by means of which the current fermentation-tube results may be converted into numerical values. The justification of McCrady's method has been amply proved, but, heretofore, their widespread use has been restricted on account of the cumbersome calculations involved when any but recurring series of tube dilutions are employed and also by the inherent dislike of most bacteriologists for apparently involved mathematical deductions.

Inasmuch as we were seeking an interpretation of qualitative results which would give numbers of bacteria which might aproach a greater degree of precision than the usual method of "averages" now commonly employed, some study was made of the methods proposed by

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[1] Am. Pub. Health Assoc., Report 33, p. 9.

[2] Jour. Infect. Dis., 1915, 17, p. 183.

McCrady in the hope of so modifying them as to make their application simple and consequently of greater frequency.

The formula proposed by McGrady for the calculation of the most probable number of B. coli is as follows:

$$(p+q)\ (\log .9) + (r+s)\ (\log .99) + (t+u)\ (\log .999) = \frac{p\ (\log 0.9)}{1-0.9^x} + \frac{r\ (\log 0.99)}{1-0.99^x} + \frac{t\ (\log .999)}{1-.999^x}$$

in which $(p+q)$, $(r+s)$, and $(t+u)$ are the total number of tubes inoculated in 10, 1, and 0.1 c.c. dilutions, while p, r, and t are the number of tubes found to be positive in each of the above series.

The solution of the above equation involves the trial substitution of possible values of "x" (the most probable number of bacteria per 100 c.c.) until the equation is found to balance. The corresponding value of "x" for this condition is the value we are seeking for any possible combination of fermentation-tube results. In examining the equation, we find that:

$$\begin{aligned}\log .9 &= .04576\\ \log .99 &= .00436\\ \log .999 &= .000435\\ \log .9999 &= .0000434\end{aligned}$$

For practical purposes, it may be stated, therefore, that approximately

$$\log .9 = 10 \log .99 = 100 \log .999 = 1000 \log .9999$$

and the formula above reproduced becomes, with this substitution:

$$100\ (p+q) + 10\ (r+s) + 1\ (t+u) = \frac{100\ p}{1-.9^x} + \frac{10\ r}{1-.99^x} + \frac{t}{1-.999^x}$$

knowing the possible values of $(p+q)$, $(r+s)$, $(t+u)$, p, r, and t, it becomes a matter of little mathematical calculation to establish the value of "x" by trial solutions.

In order to make the solution of this modified formula still more practical and more facile, the values of $1-.9^x$, $1-.99^x$, and $1-.999^x$ were graphically plotted on the attached chart. The curves, correlating values of "x" with the values of $1-.9^x$ etc., are exponential curves having exactly the same form and value as those given by McCrady, showing the most probable number of B. Coli per 100 c.c. with varying percentages of samples showing positive tests in 10, 1, 0.1 c.c. samples, respectively. By using the two scales indicated in our charts, it is made to serve two purposes; firstly, the interpretation of single dilution results, and, secondly, readings for actual calculations of values of $1-.9^x$ etc., for possible trial values of "x." The accuracy of these

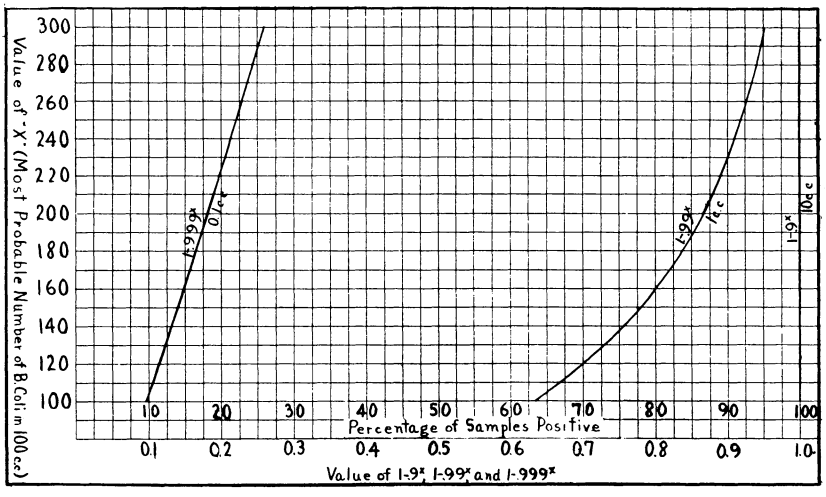

Chart 1.—Chart showing values of "X" (the most probable number of B. coli per 100 c.c.), and 1—.9×, 1—.99× and 1—.999× for varying conditions.

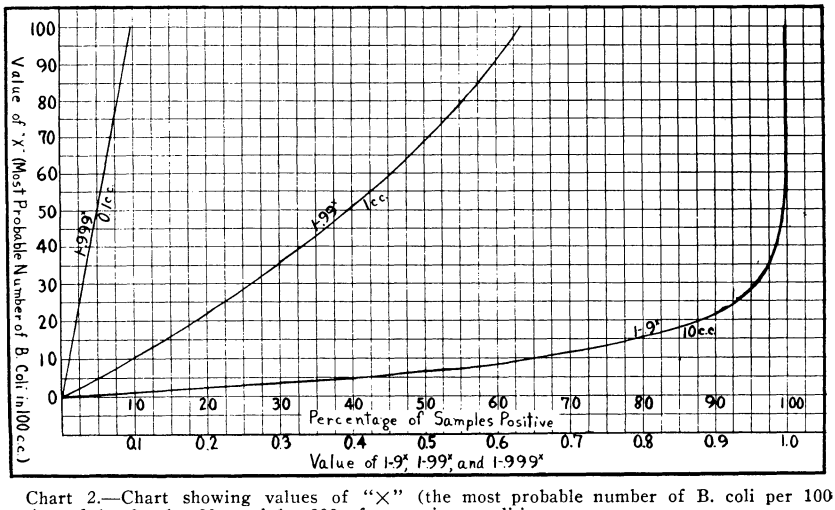

Chart 2.—Chart showing values of "X" (the most probable number of B. coli per 100 c.c.), and 1—.9×, 1—.99× and 1—.999× for varying conditions.

charts can, of course, be made as high as desired, and it is McCrady's opinion (by letter) that the error involved in the use of the curves is slight and that the method is certainly sufficient for practical use, especially for the ordinary ranges of the value of "x."

For purposes of clarification of the above method, it is well to solve several typical examples, as follows:

Single Dilution: Given the result 4 out of 5 positive in 10 c.c., to find the most probable number of B. coli in 100 c.c.

4/5 in 10 c.c. is equivalent to 80% positive.

Using this value of 80 as an ordinate, with the 10 c.c. curve on Chart 1, we find the corresponding value of the abscissa to be 15. The most probable number of B. coli per 100 c.c. is, therefore, 15, for the condition 4/5 in 10 c.c.

Several Dilutions: Suppose the result of the fermentation tubes gives positive values of ½ in 10 c.c., 3/10 in 1 c.c., and 1/10 in 0.1 c.c., the above equation becomes:

$$100\ (2) + 10\ (10) + 1\ (10) = 310 = \frac{1\ (100)}{1 - .9^x} + \frac{10\ (3)}{1 - .99^x} + \frac{1}{1 - .999^x}$$

Using trial value of $x = 22$, by referring to the chart we have:

$$1 - .9^x = .90 \quad 1 - .99^x = .195 \quad \text{and} \quad 1 - .999^x = .02$$

$$\text{Therefore: } 310 = \frac{100}{.90} + \frac{30}{.195} + \frac{1}{.02} = 315$$

Using trial value of $x = 23$:

$$1 - .9^x = .91 \quad 1 - .99^x = .205 \quad 1 - .999^x = .02$$

$$\text{Therefore: } 310 = \frac{100}{.91} + \frac{30}{.205} + \frac{1}{.02} = 308$$

The probable number of B. coli per 100 c.c. is, therefore, between 22 and 23.

It has probably already become clear to the reader that the divisions carried out above may be performed generally with sufficient accuracy by means of the slide rule.

In order to determine the validity of the assumption that $\log .9 = 10 \log .99 = 100 \log .999$, it is necessary to eliminate the error due to picking values of $1 - .9^x$, $1 - .99^x$, and $1 - .999^x$ from the curve. This was suggested to us by McCrady and was carried out, using accurate tables of $1 - .9^x$, etc. The calculations follow:

Fermentation Tube Results	Trial Values of x	Reduced Eq. Exact McC.	Reduced Eq. Modified W. & W.	Probable No. B.Coli Per 100 C.C. McC.	Probable No. B.Coli Per 100 C.C. W. & W.
1/2 3/10 1/10.....	21	316	310	21	22
	22	308			
0/2 2/10 0/10.....	6	342	310	6	7
	7	295			
2/2 5/10 2/10.....	85	312	310	86	87
	90	307			
2/2 10/10 4/10.....	540	310.02	310	550	540
	550	309.85			
2/2 3/10	35	306	300	37	37
	40	293			
2/2 1/10	17	304	300	18	17
	18	295			
2/2 1/2 0/2.....	...	...	222	61	60
2/2 1/5	...	...	250	27	28
2/2 3/5 2/5.....	...	...	255	140	138
Average				105	104

It is evident, from these figures, that the error due to the assumption under discussion is very small and in routine determinations is quite negligible. It is hoped, therefore, that the modification of McCrady's formula, because of the simplified operations involved in its solution and because of its accuracy within the usual practical limits, may lead to a wider use of the exact interpretation of fermentation-tube results in routine bacteriologic analyses.